ORIGINAL ARTICLE

Spinal antinociceptive action of amiloride and its interaction with tizanidine in the rat formalin test

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BACKGROUND: Amiloride has been reported to produce a wide variety of actions, thereby affecting several ionic channels and a multitude of receptors and enzymes. Intrathecal α_2-adrenergic receptor agonists produce pronounced analgesia, and amiloride modulates α_2-adrenergic receptor agonist binding and function, acting via the allosteric site on the α_2A-adrenergic receptor.

OBJECTIVES: To investigate the antinociceptive interaction of intrathecal amiloride and the α_2-adrenoceptor agonist tizanidine using a rat formalin test.

METHODS: Sprague-Dawley rats were chronically implanted with lumbar intrathecal catheters and were tested for paw flinching using formalin injection. Biphasic painful behaviour was recorded. Amiloride, tizanidine or an amiloride-tizanidine mixture was administered 10 min before formalin injection. To characterize any interactions, isobolographic analysis was performed. The effects of a pretreatment using intrathecally administered yohimbine was also tested.

RESULTS: Intrathecally administered amiloride (12.5 µg to 100 µg) and tizanidine (0.5 µg to 5 µg), given separately, produced a significant dose-related suppression of the biphasic responses in the formalin test. Isobolographic analysis revealed that the combination of intrathecal amiloride and tizanidine synergistically reduced phase I and II activities. Intrathecally administered yohimbine antagonized or attenuated the antinociceptive effect of amiloride, tizanidine and the amiloride-tizanidine mixture. Intrathecally administered amiloride synergistically interacts with tizanidine to reduce the nociceptive response in the formalin test, most likely by activating α_2-adrenoceptors in the spinal cord.

CONCLUSIONS: Although intrathecal tizanidine produced pronounced analgesia, antinociceptive doses of intrathecal tizanidine also produced several side effects, including bradycardia and sedation. Amiloride produced antinociceptive action against the thermal nociceptive test without side effects in rats.

Key Words: α_2-adrenergic receptor; Amiloride; Formalin test; Spinal cord

L'effet antinociceptif de l'amiloride sur la moelle épinière et son interaction avec la tizanidine dans le test au formol chez des rats

HISTORIQUE : L'amiloride produit un vaste éventail d'effets qui touchent plusieurs canaux ioniques et une multitude de récepteurs et d'enzymes. L'agoniste des alpha-2 récepteurs adrénergiques par voie intrathécale produit une analgésie prononcée, et l'amiloride en module la liaison et la fonction par son action allostérique sur le récepteur alpha-2A adrénergique.

OBJECTIFS : Examiner l'interaction antinociceptive de l'administration par voie intrathrécale d'amiloride et de tizanidine, un agoniste alpha-2 adrénergique, au moyen du test au formol chez des rats.

MÉTHODOLOGIE : Les chercheurs ont implanté un cathéter lombaire à demeure dans l'espace intrathécal de rats Sprague-Dawley et ont vérifié le tressaillement de leur patte au moyen d'une injection de formol. Ils ont consigné l'évolution biphasique de la douleur. Ils leur ont administré de l'amiloride, de la tizanidine ou un mélange d'amiloride et de tizanidine dix minutes avant de leur injecter du formol. Pour caractériser les interactions, ils ont effectué une analyse isobolographique. Ils ont également observé les effets de l'administration préalable de yohimbine par voie intrathécale.

RÉSULTATS : Dans le test au formol, l'amiloride (12,5 µg à 100 µg) et la tizanidine (0,5 µg à 5 µg), administrées séparément par voie intrathécale, produisaient une importante suppression des réponses biphasiques liées à la dose. L'analyse isobolographique a révélé que le mélange d'amiloride et de tizanidine par voie intrathécale réduisait les activités de phase I et II. L'administration de yohimbine par voie intrathécale entravait ou atténuait l'effet antinociceptif de l'amiloride, de la tizanidine et du mélange d'amiloride et de tizanidine. L'administration d'amiloride par voie intrathécale a une action synergique sur la tizanidine et en réduit la réponse nociceptive dans le test au formol, probablement en activant les alpha-2 adrénorécepteurs de la moelle épinière.

CONCLUSIONS : Même si la tizanidine par voie intrathécale produisait une analgésie prononcée, des doses antinociceptives s'associaient à plusieurs effets secondaires, y compris la bradycardie et la sédation. L'amiloride produisait un effet antinociceptif sur le test nociceptif thermique, sans provoquer d'effets secondaires chez des rats.

Amiloride is known to act on pharmacological target sites that are implicated in pain processing by inhibiting targets such as low-threshold Ca^{2+} channels at a low concentration (1), Na^+/Ca^{2+} exchange systems at a higher concentration (2), Na^+/H^+ exchangers (2), biosynthetic pathways for proinflammatory cytokines (3), ATP-sensitive K^+ channels (4), adenosine A_1 receptors (5), the gamma-aminobutyric acid-A receptor complex (6) and H^+-gated cation channels (7). Additionally, amiloride has proven to be a useful drug for studying the molecular mechanisms involved in Na^+ translocation across cell membranes (8). The systemic, spinal or supraspinal administration of amiloride causes dose-related antinociception, as indicated by assessing acetic acid-induced writhing and formalin- and capsaicin-induced licking (9). The mechanism by which amiloride produces its antinociceptive action appears to be unrelated to nonspecific toxic

effects, such as muscle relaxation or sedation in animals, and appears to be associated, at least in part, with the interference of homeostasis and function of the somatosensory system. Thus, amiloride or its derivatives may be of potential interest in the development of new antihyperalgesic drugs. The α_2-adrenoceptor agonist is widely used for analgesia and sedation. Intrathecal α_2-adrenoceptor agonist has been revealed to produce pronounced analgesia (10). Tizanidine is a novel, centrally acting α_2-adrenoceptor agonist that is available in oral form for the treatment of muscle spasms (11), and also has significant antinociceptive effects without hypotension in rats when administered intrathecally (12,13) or subcutaneously (14). Although tizanidine is associated with a moderate incidence of sedation when administered at effective systemic doses, it is well tolerated clinically, with a low incidence of hemodynamic side effects (15).

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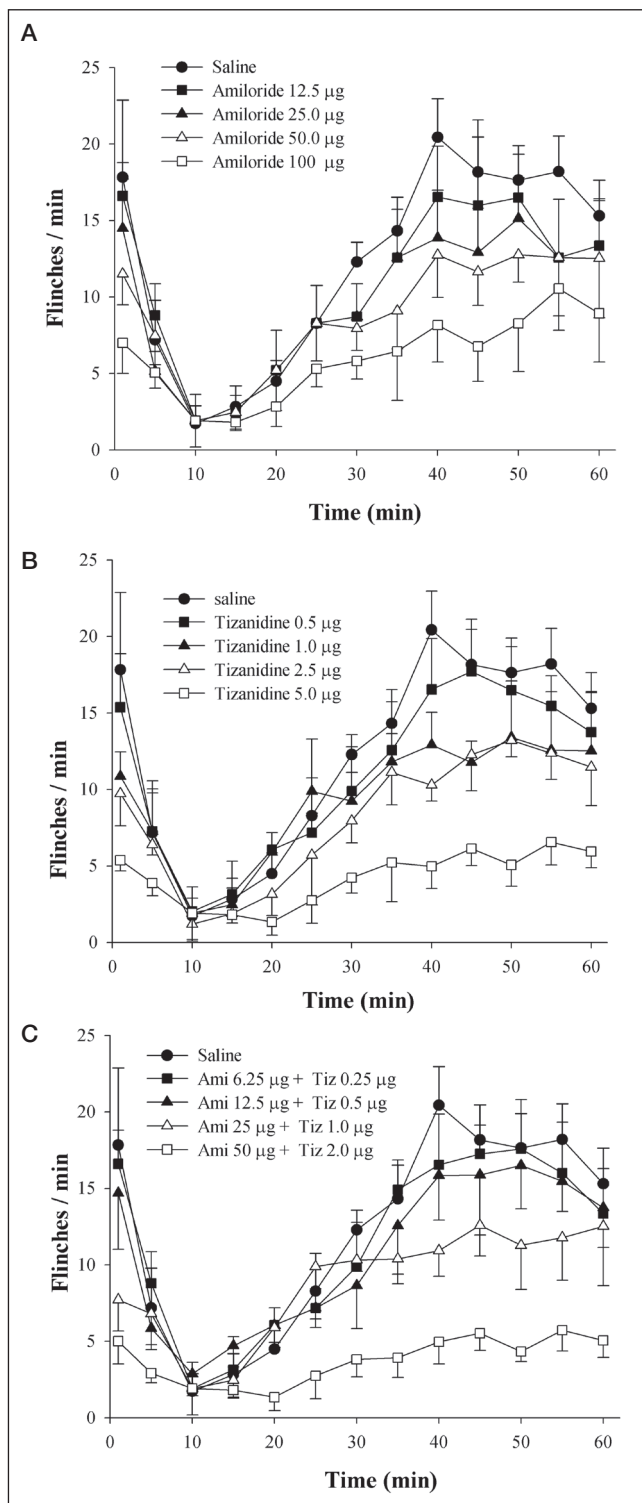
Figure 1) *Time-effect curve of intrathecal amiloride (Ami) (**A**), tizanidine (Tiz) (**B**) and amiloride and tizanidine combinations (**C**) administered before formalin. The number of flinches per min is plotted versus the time after the formalin injection into the hindpaw. Each line on the graph represents the mean ± SD from six rats*

In a previous study, we found that the intrathecal administration of amiloride produced significantly dose-dependent antinociception in the thermal tail-flick test, and a synergistic interaction exists between amiloride and morphine or clonidine (16). Leppik and Birdsall (17) revealed that amiloride modulated α_2-adrenergic receptor

agonist binding and function, acting via the allosteric site on the α_2A-adrenergic receptor. Amiloride competes for the agonist binding site on the α_2A-adrenergic receptor at low concentrations (17). However, no study has investigated whether amiloride produces antinociception via the α_2-adrenergic receptor against formalin-induced pain.

Drug combinations are an alternative approach used to obtain effective analgesia, while decreasing the incidence and severity of side effects. There has been increasing research in recent years examining drug combinations that produce analgesia in experimental animals, which may be useful in clinical research. The general purposes for using a combination of drugs are to enhance analgesia through either synergism or additive effects, and to reduce the side effects of the drugs by either reducing the dose of each drug or allowing the drugs to interact so that one drug reduces the side effects of the other. To the best of our knowledge, no study has evaluated the effectiveness of the antinociceptive interaction of amiloride and tizanidine in the formalin test. Accordingly, the purpose of the current study was to assess the interaction between intrathecal amiloride and tizanidine in the formalin test.

METHODS

Animals
The present research was approved by the Animal Care and Use Committee of Sun Yat-Sen University (Guangzhou, China). Studies were performed using male Sprague-Dawley rats weighing 250 g to 300 g. Rats were housed individually in a temperature-controlled (21±1°C) room with a 12 h light/dark cycle, and were given ad libitum access to water and food. All surgical procedures were performed with the rats under intraperitoneally administered 10% chloral hydrate (3 ml/kg to 4 ml/kg) anesthesia. Using the method described by Ouyang et al (16), an intrathecal catheter (PE-10, 8.5 cm) was inserted through an opening in the cisterna magna to the lumbar subarachnoid space. Animals showing neurological deficits after implantation were excluded. After surgery, the animals were allowed to recover for one week before drug administration. Each animal was studied only one time during the experiment. After the experiment, each rat was euthanized with an overdose of pentobarbital, and an injection of 1% methylene blue was given to confirm the position of the catheter and the likely spread of the injection.

Drugs and injections
The drugs administered during the experiments were amiloride hydrochloride hydrate (molecular weight [MW] 266.09; Sigma-Aldrich, USA), tizanidine hydrochloride (MW 290.17; Sigma-Aldrich, USA) and yohimbine hydrochloride (MW 390.90; Sigma-Aldrich, USA). All drugs were dissolved in normal saline. Amiloride and tizanidine were each intrathecally administered 10 min before the formalin test. To assess the dose dependency and time courses of the antinociceptive action of intrathecal amiloride, tizanidine and coadministered amiloride-tizanidine, the animals were randomly assigned to different groups receiving intrathecal injections of different doses of amiloride (12.5 μg, 25 μg, 50 μg, 100 μg), tizanidine (0.5 μg, 1 μg, 2.5 μg, 5 μg) or amiloride plus tizanidine 10 min before formalin injection. In the control group, intrathecal saline was given. Yohimbine was intrathecally administered 10 min before 5 μg tizanidine, 100 μg amiloride or the dose that resulted in 50% of the saline response (ED_{50})/2 of amiloride + tizanidine ED_{50}/2 mixture. The agents were delivered using a microsyringe in a total volume of 10 mL, followed by 10 mL of saline to flush the catheter.

Formalin test
For formalin injection, 50 mL of 5% formalin was subcutaneously injected into the dorsal surface of the left hindpaw using a 27-gauge needle. Animals were then placed in a clear plexiglas cylinder (20 cm × 30 cm) for observation. A mirror was placed below the floor (which was made of plexiglas) at a 45° angle to allow for unencumbered observation during the test. Within 1 min of the injection, the rat exhibited the typical behaviour of this model, ie, holding the

TABLE 1
Intrathecally administered amiloride, tizanidine or their combination in the formalin test

Group	Phase I					Phase II				
	Amiloride component		Tizanidine component			Amiloride component		Tizanidine component		
	Intrathecal dose	Fraction of ED$_{50}$	Intrathecal dose	Fraction of ED$_{50}$	TFV	Intrathecal dose	Fraction of ED$_{50}$	Intrathecal dose	Fraction of ED$_{50}$	TFV
Amiloride	92.7 (69–116)	1.00	—	—	1.00	103.0 (92–116)	1.00	—	—	1.00
Tizanidine	—	—	3.78 (3.12–4.40)	1.00	1.00	—	—	3.76 (3.53–3.99)	1.00	1.00
Amiloride + tizanidine	16.3 (10.9–21.9)	0.18	0.65 (0.43–0.88)	0.17	0.35	18.8 (14.6–22.9)	0.18	0.75 (0.58–0.92)	0.20	0.38

Data presented as effective dose (µg) resulting in a 50% reduction of control response (ED$_{50}$) (95% CI) unless otherwise indicated. Total fraction value (TFV) <1 indicates a synergistic interaction; TFV >1 indicates a antagonistic interaction; TFV=1 indicates an additive interaction.

injected paw slightly above the floor. During this period, spontaneous flinching of the injected paw could also be observed. Flinching was readily discriminated and was characterized as a rapid and brief withdrawing or flexing of the injected paw. Pain-related behaviour was quantified by counting the number of flinches for 1 min periods from 1 min to 2 min and 5 min to 6 min, and then at 5 min intervals during the period from 10 min to 60 min after the formalin injection. Two phases of spontaneous flinching behaviour were observed. An initial acute pain response (phase I, during the 0 min to 6 min interval after the formalin injection) was followed by a relatively short quiescent period, and then by a prolonged tonic response (phase II, beginning approximately 10 min after the formalin injection). The criteria for exclusion from the study included incomplete formalin injection or excessive bleeding from the injection site.

Motor blockage was graded according to the scale proposed by Langerman et al (18) for rabbits, which was modified to the rat model as follows: 0 = free movement of the hindlimbs without limitation; 1 = limited or asymmetrical movement of the hindlimbs to support the body and walk; 2 = inability to support the back of the body on the hindlimbs, with detectable movement of the limbs and response to a pain stimulus; and 3 = total paralysis of the hindlimbs.

Statistical analyses
In the formalin test, time-response data are presented as the mean ± SD per min. The dose-response lines for the phase I and phase II effects were then fitted using least-squares linear regression analysis, and the ED$_{50}$ and their 95% CI were calculated. To calculate the ED$_{50}$ values for each drug, the number of flinches were converted into a percentage of the control using the following formula:

$$\% \text{ of control} = \frac{\text{Sum of phase I (or II) count with drug}}{\text{Sum of phase I (or II) count, control}} \times 100$$

Isobolographic analysis for the drug-drug interaction was conducted according to the procedure described by Tallarida et al (19). The method was based on comparisons of doses that were determined to be equieffective. To perform the isobolographic analysis, amiloride and tizanidine were administered in combination as fixed ratios of the equieffective ED$_{50}$ dose for each drug.

Because no significant differences were observed between the ED$_{50}$ values for both phases of the formalin test, subsequent experiments were based on one ED$_{50}$ value to evaluate two phases of the formalin test. Thus, a dose-response curve was constructed from the concurrent delivery of the two routes of administration in a constant dose ratio of ED$_{50}$ values (amiloride ED$_{50}$/2 + tizanidine ED$_{50}$/2, amiloride ED$_{50}$/4 + tizanidine ED$_{50}$/4, amiloride ED$_{50}$/8 + tizanidine ED$_{50}$/8, amiloride ED$_{50}$/16 + tizanidine ED$_{50}$/16). The dose-response curve of the combined drugs was used to calculate the actual (experimental) ED$_{50}$ value and CI. To evaluate the nature of any site-site antinociceptive interaction between tizanidine and amiloride, isobolograms were drawn by plotting the experimentally determined ED$_{50}$ value of tizanidine on the x axis and that of amiloride on the y axis, considering the drugs delivered separately and in combination.

The theoretically additive ED$_{50}$ value, assuming simple additivity, and CI were calculated according to Tallarida (20). To further describe the magnitude of the interaction, a total fraction value was calculated according to the formula:

$$\text{Total fraction value} = \frac{\text{ED}_{50} \text{ dose in combination of drug 1}}{\text{ED}_{50} \text{ value for drug 1 given alone}} + \frac{\text{ED}_{50} \text{ dose in combination of drug 2}}{\text{ED}_{50} \text{ value for drug 2 given alone}}$$

The fractional values indicated which portion of the single ED$_{50}$ value was accounted for by the corresponding ED$_{50}$ value for the combination. Values of approximately 1 indicated an additive interaction, values >1 implied an antagonistic interaction and values <1 indicated a synergistic interaction. Dose-response data were analyzed using a one-way ANOVA with Bonferroni analysis and were compared with the saline control group. For statistical comparison of the difference between the theoretical additive point and the experimentally derived ED$_{50}$ value, Student's t tests were used; P<0.05 was considered to be statistically significant.
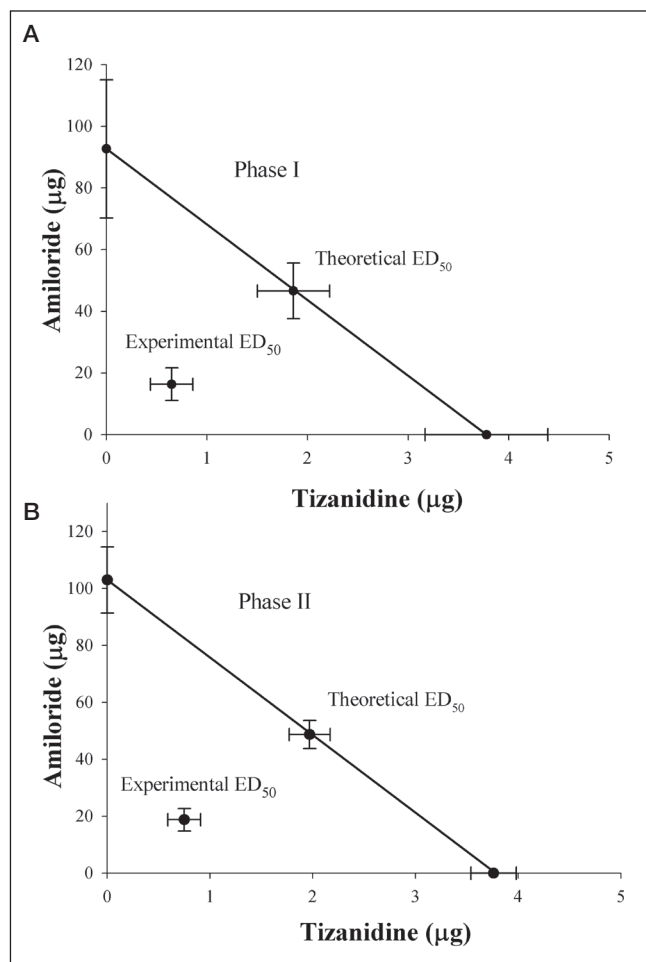
RESULTS
Individual drug responses
The subcutaneous injection of formalin into the hindpaw resulted in a biphasic flinching response of the injected paw. The timing or magnitude of the behavioural response did not differ among the control experiments (intrathecal saline 10 min before formalin; one-way ANOVA; P>0.05); thus, the control experiments were pooled and used as a common control group.

The dose-dependent effects of intrathecal amiloride and tizanidine separately produced a suppression of the formalin-induced behavioural response (Figure 1). The ED$_{50}$ values of amiloride in phases I and II were 92.7 µg (95% CI 69 µg to 116 µg) and 103.0 µg (95% CI 92 µg to 116 µg), respectively. The ED$_{50}$ values of tizanidine in phases I and II were 3.78 µg (95% CI 3.12 µg to 4.40 µg) and 3.76 µg (95% CI 3.53 µg to 3.99 µg), respectively.

Isobolographic analyses
The isobologram of amiloride and tizanidine combined revealed that the experimentally derived ED$_{50}$ values of amiloride and tizanidine in phase I were 16.3 µg (95% CI 10.9 µg to 21.9 µg) and 0.65 µg (95% CI 0.43 µg to 0.88 µg), respectively. The theoretical additive points of amiloride and tizanidine in phase I were 1.86 µg (95% CI 1.50 µg to 2.22 µg) and 46.58 µg (95% CI 37.5 µg to 55.6 µg), respectively. The experimentally derived ED$_{50}$ values of amiloride and tizanidine in phase II were 18.8 µg (95% CI 14.6 µg to 22.9 µg) and 0.75 µg (95% CI 0.58 µg to 0.92 µg), respectively. The theoretical additive points of amiloride and tizanidine in phase II were 1.97 µg (95% CI 1.77 µg to 2.12 µg) and 48.7 µg (95% CI 43.7 µg to 53.7 µg), respectively. The experimentally derived ED$_{50}$ values decreased below the theoretical dose-additive line, and the CIs of the theoretical additive points and those of the experimental points did not overlap (Figure 2). The total fraction values in phases I and II were 0.35 and 0.38, respectively (Table 1). These results indicated a significant difference between

Figure 2) *Isobologram revealing the interaction between intrathecal amiloride and tizanidine in phases I and II of the formalin test. The dose that resulted in 50% of the saline response (ED$_{50}$) values of amiloride and tizanidine are plotted on the x- and y-axes, respectively. The line connecting the ED$_{50}$ points is the theoretical additive line and the theoretical additive point for the drug combination is shown on the additive line. The experimental ED$_{50}$ value of the combination of the two agents was significantly lower than the theoretical additive value (P<0.05), indicating a synergistic interaction*

the experimental ED$_{50}$ point and the theoretical additive ED$_{50}$ point (P<0.05), and a synergistic interaction between amiloride and tizanidine in phases I and II of the formalin test. Intrathecal pretreatment with yohimbine (20 μg) antagonized or attenuated the effects of amiloride (100 μg), tizanidine (5 μg) and the amiloride-tizanidine mixture in phases I and II (Figure 3). Yohimbine (20 μg) alone did not show any antinociception in the formalin test in our research (Figure 4).

Other effects
The assessment of motor functions revealed no differences in scores on the modified scale (see Methods), regardless of whether the observations were made before or after intrathecally administering amiloride or tizanidine during the observation period. Similarly, no motor impairment was observed in the animals after the intrathecal administration of the amiloride and tizanidine combination (Table 2).

DISCUSSION
There were two important observations in our study. The intrathecal administration of amiloride and tizanidine produced a significant dose-antinociceptive effect in the formalin test. Additionally, a synergistic effect was observed between amiloride and tizanidine with regard to spinally mediated formalin pain via the α$_2$-receptor.
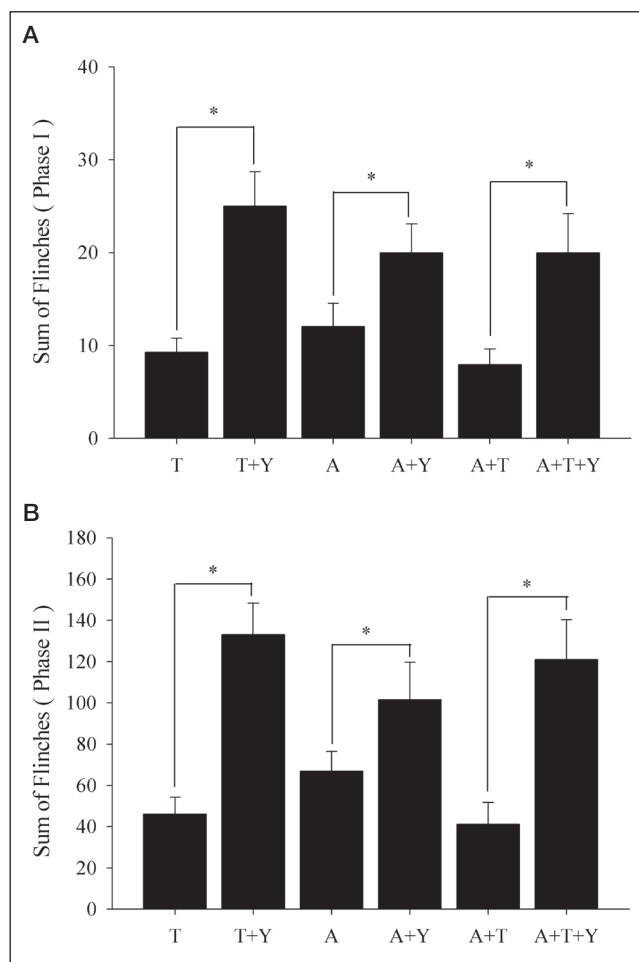
Figure 3) *To examine pharmacological antagonism of the effects of amiloride (A; 100 μg), tizanidine (T; 5 μg) and amiloride (50 μg)-tizanidine (2 μg), yohimbine (Y; 20 μg) was intrathecally administered 10 min before the administration of the agonists. Each bar represents the sum of flinches in phase II (mean ± SD) from six rats (*P<0.05)*

Although the mechanisms for amiloride antinociception have not been completely established, several hypotheses have been proposed. Amiloride has been reported to produce a wide variety of actions affecting several ion channels and a multitude of receptors and enzymes (5,21,22), such as by inhibiting Na+ transport in a wide variety of cellular systems and blocking T-type calcium channels (1). The systemic, spinal or supraspinal administration of amiloride at doses that do not cause side effects (motor dysfunction) causes dose-related antinociception, assessed in terms of acetic acid-induced writhing and formalin- and capsaicin-induced licking (9).

Dose-dependent amiloride inhibited acid-induced mechanical hypersensitivity in male mice (23), and reversed both thermal and mechanical hypersensitivity produced by inflammation or skin incision (24). The injection of amiloride was found to significantly attenuate acid-mediated pain, suggesting that amiloride-sensitive acid-sensing ion channels are responsible for a substantial proportion of acid-mediated pain (25). The amiloride-sensitive Na+-channel/degenerin family constitutes a group of proteins that is thought to be involved in a wide variety of functions, such as sodium and pH homeostasis, mechanical stimulus transduction and nociception (26). All of these effects may at least partially contribute to the reported antinociceptive action of amiloride. Leppik and Birdsall (17) revealed that amiloride modulated α$_2$-adrenergic receptor agonist binding and function, acting via the allosteric site on the α$_2$A-adrenergic receptor. Amiloride may have

TABLE 2
Motor blockage after intrathecally administered amiloride, tizanidine or their combination in the formalin test

Groups	Time, min												
	1	5	10	15	20	25	30	35	40	45	50	55	60
Amiloride 100 µg	0	0	0	0	0	0	0	0	0	0	0	0	0
Tizanidine 5.0 µg	0	0	0	0	0	0	0	0	0	0	0	0	0
Amiloride 50 µg + tizanidine 2 µg	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = free movement of the hindlimbs without limitation

competed for the agonist binding site on the α_2A-adrenergic receptor at low concentrations (17). However, evidence indicates that local peripheral or intrathecal pretreatment but not post-treatment with amiloride increased long-lasting secondary allodynia and hyperalgesia in both hindpaws in the formalin test (27). The spinal administration of amiloride augmented formalin-induced flinching behaviour. The pronociceptive effect of amiloride observed in the formalin test may be attributable to changes in the intracellular pH induced by the inhibition of the spinal Na^+/H^+ exchanger 1 (28). The reason for this discrepancy is unclear, but may be related to the amiloride concentration and dosage because different amiloride concentrations and dosages exert different actions (1,2). In our study, amiloride produced a significant dose-dependent antinociceptive effect in the formalin test during phases I and II, and this effect was partly attenuated by the α_2-adrenergic blocker yohimbine.

An intrathecal α_2-adrenoceptor agonist has been shown to produce pronounced analgesia (10). The α_2-adrenoceptor agonist is widely used for analgesia and sedation. Tizanidine is a novel, centrally acting α_2-adrenoceptor agonist that is available in an oral form for the treatment of muscle spasms (11), and also has significant antinociceptive effects without hypotension in rats when given intrathecally (12,13) or subcutaneously (14). Tizanidine was injected into the paw and produced a dose-dependent antinociceptive effect against formalin-induced pain. Isobolographic analyses revealed a significant antagonistic interaction in the tizanidine-tramadol combinations (29). Although tizanidine is associated with a moderate incidence of sedation when administered at effective systemic doses, it is well tolerated clinically, with a low incidence of hemodynamic side effects (15). Although oral tizanidine is classified therapeutically as a muscle relaxant, it does not suppress monosynaptic and polysynaptic reflexes when administered intrathecally (12). Previous reports have suggested that tizanidine produced antinociceptive effects in tail-flick tests at dose levels that were considerably lower than those expected to show muscle relaxant action (12,30). These antinociceptive effects were also reversed by the α_2-adrenergic antagonist yohimbine (12). In the current study, the doses of amiloride and tizanidine used did not show any changes in motor function scores and attenuated both phases of the flinching response, and these actions were antagonized or attenuated by yohimbine administration. This finding indicates that the action of amiloride is at least partly via an α_2-adrenergic receptor.

The interaction mechanism between the two different types of drugs may be that the different receptors localized on individual primary afferent neurons are coupled to a single class of ion channels. Synergistic interactions can occur when drugs affect different critical points along a common pathway (31). Therefore, the simultaneous activation of a common, second messenger pathway within individual neurons by two different receptors may facilitate the effector mechanisms. Amiloride can modulate α_2-adrenergic receptor agonist binding and function, acting via the allosteric site on the α_2A-adrenergic receptor (17). In the current study, the action of the amiloride-tizanidine mixture was antagonized by yohimbine, suggesting that the synergistic effects may be at least partly mediated by α_2-adrenoceptor, but not by the muscle-relaxant properties or the other pharmacological actions of tizanidine or amiloride. Furthermore, one may conclude that the synergistic effects of amiloride and tizanidine may block these channels. Na^+/H^+ exchange may be another common pathway for amiloride and
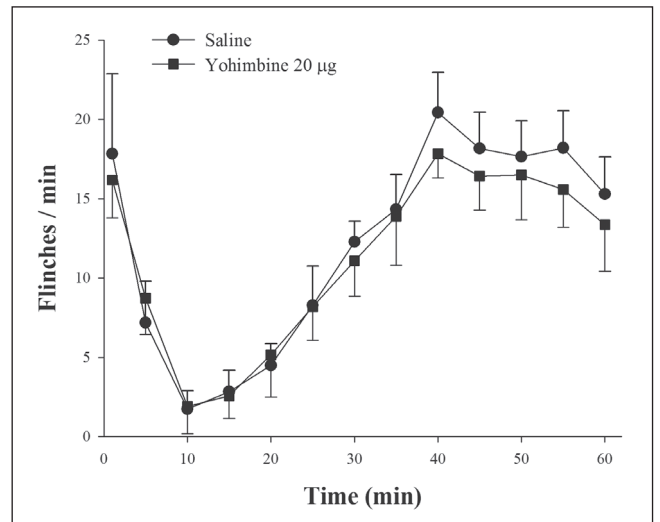

Figure 4) *Time-effect curve of the intrathecal administration of 20 µg yohimbine before formalin. The number of flinches per min is plotted versus the time after the formalin injection into the hindpaw. Each point on the plot represents the mean ± SD from six rats*

tizanidine (2,32). Whether the pharmacokinetic variables of one drug may be altered by combination with a second drug remains unclear. Although we did not study the redistribution of the intrathecal drugs, Monasky et al (33) reported that spinal α_2-adrenoceptor agonists did not alter the spinal clearance of other spinal drugs. Therefore, it seems unlikely that the synergistic interaction between tizanidine and amiloride depends on the altered clearance of either drug. As a limitation of the present study, we did not investigate all of the possible mechanisms underlying the observed synergism; this study demonstrated only that the intrathecal administration of amiloride and tizanidine had a synergistic effect against formalin-induced pain in both phases via the α_2-adrenergic receptor. Additional mechanisms should be investigated, and this type of synergistic interaction in a drug mixture may be useful in clinical pain therapy.

CONCLUSION

The current study revealed that intrathecal amiloride and tizanidine produce antinociceptive effects in a dose-dependent manner in the formalin test, and that the antinociceptive, synergistic interaction observed between amiloride and tizanidine by isobolographic analysis is at least partly via the action of the α_2-adrenoceptor in the spinal cord.

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